TO THE NOBEL COMMITTEE AND ROYAL SWEDISH ACADEMY OF SCIENCES, STOCKHOLM, SWEDEN
TO THE SCIENTIFIC AND TO ALL AUTHORITIES WORLDWIDE
TO ACADEMIC COMMUNITY IN ROMANIA
TO MASS-MEDIA WORLDWIDE

PETITION FOR THE RECOGNITION OF GHEORGHE BENGA, AS A DISCOVERER OF THE FIRST WATER CHANNEL PROTEIN IN THE HUMAN RED BLOOD CELL MEMBRANE, SEVERAL YEARS BEFORE

In 1986, Benga and coworkers (1) clearly demonstrated for the first time the presence and location of a water channel protein in the human red blood cell (RBC) membrane among polypeptides migrating in the region of 35-60 kD on the electrophoretogram of RBC membranes, labeled with ^{203}Hg-*p*-chloromercuribenzene sulfonate (PCMBS) under conditions for the specific inhibition of water diffusion. I suggested that a minor membrane component that binds PCMBS is involved in water transport and also indicated the way in which the specific protein could be further characterized: by purification and reconstitution in liposomes. In the same year the labeling experiments were confirmed and extended (2) and in the following 2-3 years I described the novelty of our work in several reviews (3-8).

In 1988, Agre and coworkers purified a new protein from the RBC membrane (9), nick-named CHIP28 (channel-forming integral membrane protein of 28 kD) (10). However, in addition to the 28 kD component, the protein had a 35-60 kD glycosylated component, i.e, the one we detected as the binding site of PCMBS under conditions for the inhibition of water transport across the RBC membrane (1,2). They suggested that CHIP28 may play a role in the linkage of the membrane skeleton to the lipid bilayer (9).

In 1990, Parker first suggested in personal discussion to Agre that the novel protein might be the water channel, and in 1992, Agre and coworkers (11), based on Windager's suggestion to use oocyte expression as a mechanism to study water transporters, found that oocytes from *Xenopus laevis* microinjected with in vitro-transcribed CHIP28 RNA exhibited increased osmotic water permeability. The water permeability was inhibited by mercuric chloride, therefore, it was suggested that CHIP28 is a functional unit of membrane water channels. By reconstitution in liposomes it was shown that CHIP28 is a water channel itself rather than a water channel regulator. In 1993 CHIP28 was renamed aquaporin 1.

It is obvious and overwhelmingly documented from the facts presented above that the first water channel protein (aquaporin 1) was discovered in 1986 by Benga *et al* (1,2). He described one of its essential components: a molecular weight of 35-60 kD for the glycosylated component, and the way to distinguish it from other proteins (reconstitution in liposomes and measurement of water permeability). Aquaporin 1 was first purified in 1988 and its water transport property was identified in 1992 by Agre and coworkers (9,11). It is also obvious that what we identified by labeling experiments is the same protein that Agre and coworkers later purified, since they mentioned (11) that "the characteristics of CHIP28 are consistent with other known features of water channels, e.g. CHIP28 proteins in intact RBCs are impervious to proteolytic digestion (9,10), as are water channels (12)".

As Agre and coworkers cited our 1983 paper (12) it is very surprising that they never cited our landmark 1986 papers (1,2); in contrast they referred only to work by other American scientists who pointed to a non-specific "pore" that allowed for permeation of anions, cations,

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nonelectrolytes and water (13). In contrast, we strongly argued all the time that there were indeed water channels in the RBC membrane and indicated the way in which specific water channel proteins could be further characterized by purification and reconstitution in liposomes.

I continued to be very active in the field, by achieving the purification of aquaporin 1 and developing a new procedure for its quantification by densitometry of silver stained gel (14). Over the last decade, we have characterized the water permeability of RBCs from over 30 species (reviewed in 15,16); we reported a positive correlation between the water permeability values of RBCs from maternal venous blood and fetal RBCs isolated from cord blood taken at delivery. This points to a genetic basis for the determination of RBC water permeability (17).

Our landmark papers in 1986 can be compared with the first detection of a child *in utero* by ultrasonography, since we discovered one of the essential components of the "aquaporin child" (a molecular weight of 35-60 kD for the glycosylated component); we have also indicated the way to recognize him after birth (among other children of his group!): placing the isolated children in a certain environment and asking them to perform the same task (one should read: reconstitution studies in liposomes and measurement of water permeability), like aligning athletes for a running test. This was the only certain way to know that the child is really the fastest runner and not just one that is helping (by various means) another child to be the fastest runner. A "new child" was observed in 1988 by Agre and coworkers, however only in 1992 the child we first detected was recognized as having the predicted qualities.

Looking in retrospect, asking the crucial question, when was the first water channel protein aquaporin 1 discovered, a fair and clear cut answer would be: the first water channel protein, now called aquaporin 1, was identified or "seen" *in situ* in the human RBC membrane by Benga and coworkers in 1986. It was again "seen" when it was by chance purified by Agre and coworkers in 1988 and was again identified when its main feature, the water transport property, was found by Agre and coworkers in 1992.

If a comparison with the discovery of The New World of America is made, the first man who has "seen" a part, very small indeed, of The New Land was Columbus; later, others, including Amerigo Vespucci (from whom the name derived), have better "seen" a larger part of the new Continent and in the subsequent years many explorers discovered the complexity of the Americas!

I presented the complete history of the discovery of water channel proteins in an invited review (18) that was published one month before the Nobel Prize for Chemistry was awarded to Peter Agre for "the discovery of water channel proteins". It appears that our seminal contribution in 1986 was grossly overlooked by Peter Agre and also by the Nobel Committee. It is another example of mistakes in awarding Nobel Prizes, when a scientist who made the very first landmark contribution to a discovery is left aside. This is my case in regard with the discovery of the first water channel protein in the human RBC membrane.

For any scientist in the world dedicated to the truth and justice there is only one conclusion: Dr Benga's initial discovery must be properly credited by the Nobel Prize Committee.

The daily newspaper "Adevarul de Cluj" (19), this should be emphasized, has mentioned and put together for the first time the two names Benga and Agre and the possibility of awarding to both scientists the Nobel Prize for the discovery of aquaporin 1.

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I am required by law to write myself the petition. Any support from scientists from all over the world would be gratefully acknowledged.

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